JOURNAL OF VIROLOGY, July 2004, p. 6982–6994 Vol. 78, No. 13
0022-538X/04/$08.00+0 DOI: 10.1128/JVI.78.13.6982–6994.2004

Complete Genome Sequence of Lymphocystis Disease Virus Isolated from China

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Received 1 December 2003/Accepted 1 March 2004

Lymphocystis diseases in fish throughout the world have been extensively described. Here we report the complete genome sequence of lymphocystis disease virus isolated in China (LCDV-C), an LCDV isolated from cultured flounder (*Paralichthys olivaceus*) with lymphocystis disease in China. The LCDV-C genome is 186,250 bp, with a base composition of 27.25% G+C. Computer-assisted analysis revealed 240 potential open reading frames (ORFs) and 176 nonoverlapping putative viral genes, which encode polypeptides ranging from 40 to 1,193 amino acids. The percent coding density is 67%, and the average length of each ORF is 702 bp. A search of the GenBank database using the 176 individual putative genes revealed 103 homologues to the corresponding ORFs of LCDV-1 and 73 potential genes that were not found in LCDV-1 and other iridoviruses. Among the 73 genes, there are 8 genes that contain conserved domains of cellular genes and 65 novel genes that do not show any significant homology with the sequences in public databases. Although a certain extent of similarity between putative gene products of LCDV-C and corresponding proteins of LCDV-1 was revealed, no colinearity was detected when their ORF arrangements and coding strategies were compared to each other, suggesting that a high degree of genetic rearrangements between them has occurred. And a large number of tandem and overlapping repeated sequences were observed in the LCDV-C genome. The deduced amino acid sequence of the major capsid protein (MCP) presents the highest identity to those of LCDV-1 and other iridoviruses among the LCDV-C gene products. Furthermore, a phylogenetic tree was constructed based on the multiple alignments of nine MCP amino acid sequences. Interestingly, LCDV-C and LCDV-1 were clustered together, but their amino acid identity is much less than that in other clusters. The unexpected levels of divergence between their genomes in size, gene organization, and gene product identity suggest that LCDV-C and LCDV-1 shouldn't belong to a same species and that LCDV-C should be considered a species different from LCDV-1.

Lymphocystis disease was discovered early in 1874 (34), but the viral agent was not detected until 1962 (36). The lymphocystis disease virus (LCDV) has been studied by a series of morphology and ultrastructure observations (2, 3, 15, 27, 36, 47), molecular characterization analysis (5, 7, 9, 12, 29, 30), and attempts at in vitro infection and propagation (25, 35, 38, 46). LCDV has been identified as an iridovirus (7, 39) and is distributed worldwide. The resulting lymphocystis disease has been reported to occur in over 100 different fish species in seawater and freshwater (34). In recent years, lymphocystis disease has been reported to occur frequently in cultured flounder (*Paralichthys olivaceus*) in China (31, 40), and the causative agent has also been identified as LCDV-C (LCDV isolated in China) (31, 40, 46).

Iridoviridae have been subdivided into four genera, including *Iridovirus*, *Chloriridovirus*, *Ranavirus*, and *Lymphocystivirus* (26). LCDV belongs to *Lymphocystivirus* and is the type species in the genus. LCDV-1, isolated in the United States, has been extensively studied, and its genome was characterized by molecular cloning and physical mapping about 20 years ago (5, 6). The genome structure, found to be common to other iridoviruses, is circularly permuted and terminally redundant (5, 6, 28, 37). In 1997, the LCDV-1 complete genomic DNA sequence was determined. The genome is 102,653 bp in length and contains 195 open reading frames (ORFs) (33). Recently, three other genomes of vertebrate iridoviruses, those of the mandarin fish infectious spleen and kidney necrosis virus (ISKNV) (13), the tiger frog virus (TFV) (14), and salamander *Ambystoma tigrinum* virus (ATV) (19), have been fully sequenced and characterized. Because lymphocystis diseases have been reported to occur in more than 100 different fish species in seawater and freshwater worldwide (34), some differences in genome structure, gene organization, and DNA sequence may exist in the virus isolates from different fish species or from different geographic regions. To reveal the genomic characterization of LCDV-C and to perform comparative-genomics studies on iridoviruses, we initiated a project to sequence the LCDV-C genome. Here we report the LCDV-C complete genome sequence and analyze the structural differences between LCDV-C and other iridoviruses.

MATERIALS AND METHODS

LCDV-C and its viral-DNA preparation. LCDV-C used in this study was originally isolated from cultured flounder (*Paralichthys olivaceus*) with lymphocystis disease from Shandong Province of China (31). The lymphocystis tissues were sampled from the tumor-like dermal lesions of diseased fish and homogenized in phosphate-buffered saline (PBS) containing antibiotics (penicillin [100 IU ml^{-1}] and streptomycin [100 $\mu g\ ml^{-1}$]). Extracts were stored overnight at −20°C, thawed, and clarified by low-speed centrifugation, and the supernatants containing LCDV-C were ultracentrifuged in a Beckman (rotor type, SW41) at 36,000 rpm (160,000 × *g*) for 40 min. The pellet was resuspended in 1 ml of PBS and further purified by using discontinuous sucrose (20, 30, 40, and 50%) gradient centrifugation at 36,000 rpm (160,000 × *g*) for 40 min. The virus particle band was collected, and sucrose was removed by further centrifugation. The purified virus particles were used to extract the LCDV-C genomic DNA by incubating virus with 0.2 mg of proteinase K/ml–1% sodium dodecyl sulfate at

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37°C for 2 h. Then the DNA was subjected to phenol-chloroform extraction and ethanol precipitation as described previously (43, 44).

DNA sequencing. The LCDV-C genome was sequenced by a shotgun approach (41). Briefly, the viral genome DNA was randomly sheared by sonication at 0°C, and blunt ends of the sonicated fragments were generated with T4 polymerase. The DNA fragments were size fractionated by gel electrophoresis, and different-size fragments, such as 1.6 to 2.0 kb, 2.0 to 2.5 kb, and 2.5 to 3.0 kb, were extracted from the gels by a QIAEXII gel extraction kit. Then the DNA fragments were cloned into the EcoRV site of the pUC18 vector with T4 DNA ligase. After transformation into *Escherichia coli* XL-100 competent cells, the recombinant plasmid DNAs were extracted, and the cloned viral DNA fragments were sequenced in both directions with M13 universal primers and other synthesized primers according to the sequences obtained with an ABI 3700 automated DNA sequencer. A total of 2,929 sequencing reactions were performed, and 2,517 high-quality sequence fragments were assembled with InnerPeace software. The average reading frame length was about 600 bp with eightfold coverage of the whole genome. During the final stages of assembly, gaps were filled by sequencing PCR products amplified directly from the whole virus DNA with 32 oligonucleotide primers.

Computer-assisted analysis. Nucleotide and amino acid sequences, restriction enzyme patterns, and repeated sequences were compiled and analyzed with the programs of the DNASTAR software package (Lasergene). Putative ORFs were predicted one by one by finding the start codon AUG and the rest of the coding sequence with the DNASTAR software package; ORFs encoding more than 40 amino acids (120 bp) were considered putative ORFs. The putative viral genes were obtained from the putative ORFs of more than 40 codons by selecting nonoverlapping ORFs. When two ORFs overlapped, the larger ORF was generally chosen as the putative viral gene. DNA and protein comparisons with entries in the sequence databases were performed with BLAST programs (1, 24). Comparison of the homological sequence regions of LCDV-C, LCDV-1, and other iridoviruses was performed with BLAST programs. A phylogenetic tree was constructed by the MegAlign program of DNASTAR software on the basis of amino acid sequence alignment of the known major capsid protein (MCPs) of different iridoviruses, including LCDV-C, LCDV-1 (LCDV isolated from United States), CIV (Chilo iridescent virus), ISKNV, RSIV (Red Sea bream disease iridovirus), FV3 (frog virus 3), BIV (Bohie iridovirus), TFV, and EHNV (epizootic hematopoietic necrosis virus).

Nucleotide sequence accession number. The complete nucleotide sequence of the LCDV-C genome was deposited in GenBank under accession no. AY380826.

RESULTS AND DISCUSSION

Determination of the nucleotide sequence of the LCDV-C genome. The complete nucleotide sequence of the LCDV-C genome was determined by applying the whole-genome shotgun sequencing strategy. The LCDV-C genome consists of 186,250 bp (Table 1). Among the sequenced vertebrate iridoviruses, LCDV-1 is 102,653 bp (33), TFV is 105,057 bp (14), ISKNV is 111,362 bp (13), and ATV is 106,332 bp (19). LCDV-C has of the largest genome among them. However, another invertebrate iridovirus, CIV, which was analyzed by Jakob et al. (16), has a genome larger (212,482 bp) than that of the LCDV-C. The base composition of the LCDV-C genome was found to be 27.25% G+C. The low G+C ratio is similar to those of LCDV-1 (29.07%) (33) and CIV (28.63%) (16) but is significantly different from those of ISKNV (54.78%) (13), TFV (55.01%) (14), and ATV (54%) (19). Therefore, the markedly low G+C content is a characteristic of the genus *Lymphocystivirus*.

In addition, about 0.4% nucleotide replacement heterogeneity has been observed from the repeatedly sequenced LCDV-C genome sequences, and the majority of the replacements are in the noncoding regions. The polymorphism might be related to the virus materials used for sequencing, because the virus materials could be potentially heterogenous, containing sequences from a number of different variant viruses.

Organization and coding capacity of the LCDV-C genome. Computer-assisted analysis of the LCDV-C genomic DNA sequence revealed the presence of 240 potential ORFs. As shown

TABLE 1. Comparison of genome size and genome characterization for the sequenced iridoviruses

Virus	Genus	Genome size (bp)	GC content (%)	No. of		Coding density (%)	Avg length of ORF (bp)	No. of encoded amino acids	Yr determined	GenBank accession no.	Reference or source
				Potential ORFs[a]	Putative genes						
LCDV-C	*Lymphocystivirus*	186,247	27.25	240	176	67	702	40–1,193	2004	AY380826	This paper
LCDV-1	*Lymphocystivirus*	102,653	29.07	195	110	82	822	40–1,199	1997	L63545	32
CIV	*Iridovirus*	212,482	28.63	468	234	85	843	40–2,432	2001	AF303741	17
ISKNV	Undetermined	111,362	54.78	124	105	93	834	40–1,208	2001	AF371960	13
TFV	*Ranavirus*	105,057	55.01	105	105	94	873	40–1,294	2002	AF389451	14
ATV	*Ranavirus*	106,332	54	96	96	79	933	32–1,294	2003	AY150217	19

[a] Determination of the numbers of ORFs involved different criteria. See references.

TABLE 2. Potential ORFs of the LCDV-C genome and comparison to those of LCDV-1 and other iridoviruses

ORF[b]	Nucleotide position	No. of amino acids	Conserved domain or signatures	CD accession no.	Homologues to LCDV-1			Homologues to other iridoviruses in *Iridoviridae*		
					% Identity of amino acids[c]	Accession no.	Predicted function and/or similarity[a]	% Identity of amino acids	Accession no.	Species
001L	649–524	42								
002L	1661–1362	100	Caspase recruitment domain; motif contained in proteins involved in apoptotic signaling; predicted to possess a DEATH (pfam00531) domain-like fold	pfam00619.8						
003R	1911–2930	340	3-β-Hydroxysteroid dehydrogenase/isomerase family	pfam01073	60 (163/263)	NP_078739.1	Hydroxysteroid dehydrogenase; steroid D5-D4 isomerase; ORF31			
004L*	2062–1940	41								
005R	2992–3243	84								
006R	3686–4183	166			29 (26/89)	NP_078657.1	ORF67			
007L	5259–4765	165			61 (101/165)	NP_078618.1	ORF70	37 (39/103)	NP_612278.1	ISKNV
008R*	5126–5284	53						33 (35/106)	AAB94419.1	CIV
009L	6022–5741	94			68 (65/95)	NP_078638.1	VLTF2-like late transcription factor, ORF102	31 (30/94)	AF397203	Regina ranavirus
010L	6508–6371	46						31 (21/66)	AF303741	CIV
011L	7532–6675	286	Thymidylate synthase.	pfam00303.8						
012R	7733–8116	128			74 (95/128)	NP_078640.1	ORF88	36 (45/125)	AF368231	Regina ranavirus
013L	10058–8502	519	Serine/threonine protein kinases, catalytic domain; phosphotransferases; serine- or threonine-specific kinase subfamily.	smart00220.7	67 (342/509)	NP_078619.1	ORF14	28 (110/380)	AF303741	CIV
								54 (20/37)	AF303741	CIV
014L	10927–10643	95			60 (57/94)	NP_078639.1	ORF97			
015L	11410–11264	49								
016L	12480–11647	278	Tumor necrosis factor (TNF) receptor domain; superfamily of TNF-like receptor domains.	cd00185.2						
017R*	12300–12473	58								
018L	13143–12955	63			36 (22/61)	NP_078710.1	ORF130			
019R	13401–14729	443			48 (212/417)	NP_078753.1	ORF21			
020L*	13912–13793	40								
021L*	15085–14945	47								
022R	15055–15702	216			50 (108/215)	NP_078727.1	ORF52			
023R	16260–16424	55			56 (30/53)	NP_078706.1	ORF139			
024R	16464–16793	110			51 (51/100)	NP_078705.1	ORF95			
025R	17018–20068	1,017	RNA polymerase Rpb2, domain 6	pfam00562.8	75 (770/1024)	NP_078633.1	DNA-directed RNA polymerase subunit 2; ORF3	45 (519/1129)	NP_572001.1	*Rana tigrina* ranavirus; Regina ranavirus
			RNA polymerase beta subunit	pfam04563.2				46 (445/957)	AF397202	ISKNV
026L*	19483–19343	47						43 (464/1079)	NP_612256.1	
027R	20577–21164	196	Deoxynucleoside kinases (nucleotide transport and metabolism)	COG1428.1	71 (137/192)	NP_078725.1	Deoxynucleoside kinase; ORF60	28 (50/175)	NP_149606.1	Invertebrate iridescent virus 6
028L*	20902–20765	46						26 (32/119)	NP_612254.1	ISKNV
029R	21472–23208	579			62 (33/580)	NP_078643.1	ORF11			
030L*	21935–21786	50								
031L*	22136–22014	41								
032L*	22635–22426	70								
033R	23615–24976	454	Membrane-bound metallopeptidase (cell division and chromosome partitioning)	COG4942.1	55 (253/454)	NP_078746.1	ORF22			
034L	26151–25423	243			63 (149/233)	NP_078713.1	Early iridovirus protein; ORF49	32 (79/241)	CAA07475.1	EHNV
								32 (78/241)	NP_571993.1	*Rana tigrina* ranavirus
								32 (79/241)	CAA37177.1	FV3
								25 (38/147)	NP_612340.1	NP_612340.1
035R*	25820–25951	44								
036R*	26074–26265	64								

037L	26497–26372	42								
038R	26690–27619	310	Poxvirus proteins of unknown function	pfam03003.8	63 (167/261)	NP_078745.1	Membrane (myristylated) protein; ORF29	31 (82/261)	AF303741	CIV
039R	28016–28684	223			44 (98/222)	NP_078768.1	ORF48			
040R	29208–29441	78			55 (33/59)	NP_078653.1	ORF116			
041L	30804–29683	374	Ribonucleotide reductase	COG0208.1	66 (239/357)	NP_078636.1	Ribonucleotide reductase small subunit			
042L	31328–31197	44					ORF26			
043L	32783–31407	459	Iridovirus MCP	pfam04451.2	87 (402/459)	NP_044812.1	MCP	52 (246/466)	AF303741	CIV, *Rana tigrina* ranavirus
								50 (236/464)	NP_572010.1	ISKNV
								48 (224/460)	NP_612228.1	RSIV
								48 (224/460)	BAC66968.1	
044R*	31987–32133	49								
045R	33396–34670	425	Serine/threonine protein kinase catalytic domain; phosphotransferases; serine- or threonine-specific kinase subfamily	smart00220.7	37 (142/378)	NP_078619.1	ORF14	27 (96/349)	AF303741	CIV
046L*	35145–34981	55								
047R	35058–36179	374	Site-specific recombinase XerD (DNA replication, recombination, and repair)	COG4974.1						
048L*	35920–35765	52								
049R	36463–36891	143								
050R	37368–37517	50								
051L	38984–37536	483	RVT (RNA-dependent DNA polymerase)	pfam00078.8						
052R*	37894–38190	99								
053L	40118–39549	190			49 (86/175)	NP_078732.1	ORF63			
054R	40140–40667	176	Histone-like transcription factor (CBF/NF-Y) and archaeal histone	pfam00808.8	80 (142/176)	NP_078762.1	ORF62			
055L*	40661–40431	77								
056L	41699–41133	189			49 (86/175)	NP_078732.1	ORF63			
057R	41721–42020	100			46 (45/96)	NP_078731.1	ORF96			
058L	43529–42531	333	7 transmembrane receptor (rhodopsin family).	pfam00001.8						
059R	44029–45015	329			36 (120/331)	NP_078704.1	Hypothetical LCDV1 paralog family 2; ORF32			
060R	45232–45366	45								
061R	45414–45923	170								
062R	46203–46793	197	Putative DNA-binding (bihelical) motif predicted to be involved in chromosomal organization	smart00513.7	55 (110/198)	NP_078703.1	ORF58	35 (44/124)	AF368231	Regina ranavirus
063L	47011–46892	40								
064L	47515–47348	56								
065R	47542–48042	167			39 (65/166)	NP_078651.1	ORF68			
066R	48716–49072	119			48 (58/119)	NP_078634.1	ORF77			
067R	49321–50020	230			35 (93/261)	NP_078741.1	ORF41			
068L*	49791–49654	46								
069L	50841–50647	65			60 (39/65)	NP_078755.1	ORF124			
070L	51380–50925	152			35 (45/126)	NP_078671.1	Apoptosis regulation; Bcl-2 family protein; ORF81			
071L	52213–51614	200								
072R	53025–54020	332			45 (154/337)	NP_078626.1	Hypothetical LCDV1 paralog family 2; ORF33			
073R	54512–54916	135			76 (102/133)	NP_078769.1	ORF84	40 (20/50)	AAB94443	CIV
074R*	55310–55465	52								
075L	58165–55331	945	SNF2 family N-terminal domain	pfam00176.8	68 (652/947)	NP_078720.1	SW1/SNF2 family helicase; ORF4	41 (398/963)	NP_571991.1	Rana tigrina ranavirus
								46 (272/586)	AF367980	Regina ranavirus
								32 (303/946)	NP_612285.1	ISKNV
								28 (325/1139)	AF083915	CIV
076R*	57617–57739	41								

Continued on following page

TABLE 2—*Continued*

ORF[b]	Nucleotide position	No. of amino acids	Conserved domain or signatures	CD accession no.	Homologues to LCDV-1			Homologues to other iridoviruses in *Iridoviridae*		
					% Identity of amino acids[c]	Accession no.	Predicted function and/or similarity[a]	% Identity of amino acids	Accession no.	Species
077R	58523–58762	80								
078R*	59279–59452	58								
079R*	59543–59668	42								
080L	61359–58765	865	Predicted ATPase (general function prediction only)	COG3378.1	71 (616/865)	NP_078717.1	D5 family NTPase involved in DNA replication; ORF6	36 (319/885)	NP_612331.1	ISKNV
								27 (259/936)	AAB94479.1	CIV
081R*	61166–61306	47								
082R	61859–62260	134			27 (39/142)	NP_078759.1	ORF18			
083R	62853–63008	52								
084R	63034–63153	40								
085L	63384–63229	52								
086L	64011–63688	108			76 (83/108)	NP_078617.1	DNA methyltransferase; ORF51	52 (50/95)	NP_572009.1	*Rana tigrina* ranavirus
								46 (45/97)	NP_612268.1	ISKNV
087L	66826–65909	306			57 (174/302)	NP_078740.1	ORF38			
088R*	66072–66224	51								
089R	67518–67647	43								
090L	68950–68408	181								
091L	69880–69479	134			44 (53/120)	NP_078761.1	ORF76			
092R*	69604–69744	47								
093L	70333–69947	129			50 (33/65)	NP_078760.1	ORF93			
094R*	70107–70229	41								
095R	70395–71261	289								
096R	71776–71934	53								
097L	72846–72037	270								
098R*	72277–72510	78								
099L	73633–73307	109			46 (51/109)	NP_078751.1	ORF94			
100L	74643–73771	291			57 (168/293)	NP_078701.1	ORF39	31 (58/184)	AF303741	CIV
101L	75803–74982	274			22 (128/558)	NP_078764.1	Putative filamentous protein; ORF10	30 (30/97)	NP_612318.1	ISKNV
102R*	75004–75309	46								
103R*	75641–75781	47								
104L	77978–76956	341			54 (189/346)	NP_078702.1	Hypothetical LCDV-1 paralog family 2; ORF30			
105R	78572–78709	46								
106L	79313–78897	139								
107L	80761–79595	389			22 (84/372)	NP_078759.1	ORF18			
108L	80916–80767	50								
109L	81476–81324	51								
110R	81572–81934	121								
111L	83147–82530	206			58 (116/200)	NP_078668.1	Uncharacterized LCDV-1 paralog family 1; ORF56			
112R	83202–83735	178			46 (79/169)	NP_078666.1	Uncharacterized LCDV-1 paralog family 1; ORF61			
113L*	83548–83429	40								
114L	84942–84211	244			81 (199/244)	NP_078656.1	Virion assembly protein; NTPase; ORF46	55 (136/244)	NP_571992.1	*Rana tigrina* ranavirus
								55 (133/239)	NP_612345.1	ISKNV
								54 (131/242)	BAA28670.1	RSIV
								50 (125/248)	AAA43823	FV3
								41 (100/240)	AAB94422	CIV
115R	85683–85919	79	DNA-directed RNA polymerase, subunit M/transcription elongation factor TFIIS (transcription)	COG1594.1	59 (45/76)	NP_078754.1	Transcription factor SII homolog; ORF42	34 (25/72)	NP_572006.1	*Rana tigrina* ranavirus

116R	86212–87081	290						30 (102/330)	NP_612294.1	ISKNV
117L	88281–87349	311			48 (157/323)	NP_078623.1	ORF35			
118R	88741–88860	40								
119L	90542–89130	471			31 (157/496)	NP_078676.1	ORF23			
120R*	89332–89475	48								
121R	90758–91093	112								
122R	91498–91881	128			34 (44/126)	NP_078670.1	ORF85			
123L	92090–91935	52								
124R	92394–93368	325								
125R	93740–93874	45								
126R	93954–94124	57								
127L	94383–94246	46								
128L	94858–94586	91			38 (35/92)	NP_078642.1	ORF104			
129R	94922–95392	157								
130L*	95223–95092	44								
131R	96217–97197	327			21 (66/305)	NP_078702.1	Hypothetical LCDV1 paralog family 2; ORF30			
132L*	96401–96228	58								
133R	97712–98722	337			48 (53/110)	NP_078708.1	ORF69			
134L*	99272–99039	78								
135R	99061–99660	200								
136L	100198–99839	120			40 (47/117)	NP_078707.1	ORF86			
137L	101109–100627	161								
138R*	100988–101140	51								
139L	101415–101275	47								
140L	102507–101458	350			64 (233/361)	NP_078696.1	Tristetraprolin-like zinc finger protein C3H; ORF28			
141R*	102098–102247	50								
142L	102988–102554	145	Ervl/Alr family; biogenesis of Fe/S clusters involves a number of essential mitochondrial proteins.	pfam04777.2	51 (74/145)	NP_078699.1	Thiol oxidoreductase; ORF79	38 (45/116)	NP_612265.1	ISKNV
								27 (29/106)	AF303741	CIV
143R	103037–103993	319			57 (180/314)	NP_078700.1	Ariadne-2 homologue; ORF36	29 (71/237)	NP_572004.1	*Rana tigrina* ranavirus
144L*	103613–103419	65								
145R	104543–104932	130			62 (79/127)	NP_078728.1	Uncharacterized LCDV1 paralog family 1; ORF87			
146L	105550–105146	135			60 (81/134)	NP_078686.1	ORF83	27 (16/58)	NP_612232.1	ISKNV
147L	105998–105555	148			75 (110/145)	NP_078685.1	ORF75			
148L	106813–106268	182	Catalytic domain of ctd-like phosphatases	smart00577.6	58 (103/177)	NP_078678.1	Putative NIF/NLI-interacting factor; ORF64	34 (57/165)	NP_612227.1	ISKNV
								32 (58/177)	AF303741	CIV
149R	106822–107196	125			57 (70/121)	NP_078679.1	ORF80			
150R	107401–108923	506			49 (254/514)	NP_078684.1	ORF15	28 (54/192)	AF303741	CIV
151R	109535–110014	160			40 (52/130)	NP_078641.1	ORF73			
152L*	109787–109662	42								
153L	110936–110439	166			63 (104/164)	NP_078627.1	ORF71	46 (66/141)	AF303741	CIV
								56 (58/114)	NP_612308.1	ISKNV
								43 (64/147)	AF367980	Regina ranavirus
154R	112593–113570	326								
155L*	112795–112677	40								
156L*	113550–113431	40								
157R	114005–114463	153			54 (83/151)	NP_078659.1	ORF74			
158R	115020–116390	457			46 (214/461)	NP_078665.1	Myristylated membrane protein A; ORF20	28 (110/385)	AF368229	Regina ranavirus
								23 (113/477)	AAB94444.1	CIV
								23 (107/450)	NP_612229.1	ISKNV
								24 (96/385)	BAC66967.1	RSIV
								25 (51/197)	AF303741	CIV
159R	116374–116736	121			56 (68/121)	NP_078664.1	ORF89			

Continued on following page

TABLE 2—*Continued*

ORF[b]	Nucleotide position	No. of amino acids	Conserved domain or signatures	CD accession no.	Homologues to LCDV-1			Homologues to other iridoviruses in *Iridoviridae*		
					% Identity of amino acids[c]	Accession no.	Predicted function and/or similarity[a]	% Identity of amino acids	Accession no.	Species
160R	117323–118495	391			32 (133/413)	NP_078759.1	ORF18			
161L	120785–118915	624	Acetyl-coenyme A hydrolase (energy production and conversion)	COG0427.1	63 (395/622)	NP_078649.1	ORF9			
162R	120803–121969	389			61 (228/371)	NP_078648.1	Hypothetical immediate-early protein; ORF27	23 (85/360)	NP_572011.1	*Rana tigrina* ranavirus
								23 (85/360)	AF367980	Regina ranavirus
								25 (80/309)	AF303741	CIV
								25 (68/269)	AF371960	ISKNV
163L*	121300–121067	78								
164L	122674–122075	200								
165L	123025–122816	70								
166L	125548–123146	801	Herpesvirus major outer envelope glycoprotein (BLLF1); serine/threonine protein kinases, catalytic domain; phosphotransferases; serine- or threonine-specific kinase subfamily	pfam05109.2; smart00220.7	44 (258/577)	NP_078689.1	Uncharacterized conserved domain linked to protein kinase domain; ORF7			
167R*	124870–125001	44								
168L	125867–125739	43								
169R	125950–126960	337	Xeroderma pigmentosum G N and I regions (XPGN, XPGI); contains the HhH2 motif; domain in nucleases	cd00128.2	54 (182/331)	NP_078767.1	Putative XPG/RAD2-type nuclease; ORF34	36 (104/283)	NP_572012.1	*Rana tigrina* ranavirus
								33 (87/259)	AF367980	Regina ranavirus
								30 (91/295)	NP_612249.1	ISKNV
								30 (89/295)	BAA82754.1	RSIV
								27 (91/331)	AF303741	CIV
170R	127874–128008	45								
171L	128623–128483	47								
172L	130914–129274	547	Ribonucleotide reductase, alpha subunit (nucleotide transport and metabolism)	COG0209.1	74 (405/547)	Np_078756.1	Ribonucleotide reductase large subunit; ORF12			
173R	131370–134168	933	Putative lipopolysaccharide-modifying enzyme	smart00672.6	64 (468/728)	NP_078770.1	ORF8	38 (200/515)	AAB94478.1	CIV
								31 (204/651)	NP_571995.1	*Rana tigrina* ranavirus
174L*	133972–133838	45								
175R	134663–136102	480	Serine/threonine protein kinases, catalytic domain; phosphotransferases of the serine- or threonine-specific kinase subfamily	cd00180.2	54 (282/522)	NP_078677.1	Phosphotransferase; ORF81	26 (109/413)	AF303741	CIV
176R	136165–136347	61								
177R	137000–137404	135								
178L	139562–138150	471	Mn^{2+}-dependent serine/threonine protein kinase (signal transduction mechanisms)	COG3642.1	53 (249/468)	NP_078729.1	Phosphotransferase; ORF17	28 (33/117)	NP_612235.1	ISKNV
179L	140985–140065	307			37 (35/93)	NP_078708.1	ORF69			
180R	141161–142072	304								
181R	142378–143133	252			67 (169/250)	NP_078747.1	Putative replication factor and/or DNA binding/packing protein; ORF43	33 (58/172)	AF303741	CIV
									23 (36/151)	NP_612283.1
	ISKNV									
182R	143285–143524	80			67 (53/79)	NP_078695.1				
183L*	143517–143373	48								
184L	144548–144423	42								
185R	144571–146019	483			55 (272/486)	NP_078744.1	ORF16	24 (87/359)	AF303741	CIV
186R	146201–146539	113								
187R	146942–147694	251			76 (193/251)	NP_078726.1	RNase III; ORF44	45 (111/246)	NP_572005.1	*Rana tigrina* ranavirus; CIV

								30 (74/241)	AAB94459.1	ISKNV
								30 (66/216)	NP_612309.1	
188L*	147656–147462	67								
189R	148578–149099	174								
190L	149785–149483	101								
191R	150198–153776	1,193	DNA-directed RNA polymerase beta′ subunit/160-kDa subunit (transcription); RNA polymerase Rpb1, domain 5	COG0086.1; pfam04998.2	69 (820/1188)	NP_078624.1	DNA-dependent RNA polymerase, largest subunit; ORF5	43 (558/1274)	NP_571990.1	*Rana tigrina* ranavirus; ISKNV
								38 (467/1226)	NP_612250.1	
								37 (463/1226)	BAA82753.1	
192L*	150408–150283	42								
193L*	151287–151156	44								
194L*	152516–152328	63								
195L*	153063–152944	40								
196L	153897–153775	41								
197L	154686–153940	249			58 (146/248)	NP_078615.1	Proliferating cell nuclear antigen; ORF45	25 (64/247)	NP_612334.1	ISKNV
198R*	154169–154297	43								
199L*	155080–154934	49								
200R*	154959–155129	57								
201L	156417–155221	399			64 (259/400)	NP_078687.1	ORF25	29 (55/185)	AF303741	CIV
202L	157950–157021	310								
203L	161193–158389	935	DNA polymerase family B; DNA polymerase elongation subunit (family B)	pfam00136.8; COG0417.1	65 (613/930)	NP_078724.1	DNA polymerase family B; ORF5	40 (411/1007)	NP_572000.1	*Rana tigrina* ranavirus; RSIV
								37 (349/942)	BAA28669.1	ISKNV
								36 (353/959)	NP_612241.1	CIV
								32 (228/692)	AF083915	Regina ranavirus
								42 (176/414)	AF368230	
204R*	158963–159220	85								
205R	161257–161703	149			87 (36/41)	NP_078723.1	ORF90			
206R*	161818–161940	41								
207L*	161986–161849	46								
208L	163448–163005	148								
209R	163864–165933	690	Cell division protein 48 (CDC48), N-terminal domain	pfam02359.8						
			ATPase family associated with various cellular activities (AAA)	pfam00004.8						
210L*	164576–164388	63								
211R	166014–166280	89								
212L	166664–166446	73								
213L*	166783–166694	41								
214R	166685–167239	185			23 (46/188)	NP_078681.1	ORF59			
215L	167845–167714	44								
216L	168452–167850	201	Collagen triple helix repeat	pfam01391.8						
217R	168693–169028	112			51 (58/112)	NP_078751.1	ORF94			
218R	169041–169225	62			39 (23/58)	NP_078752.1	ORF121			
219R	169337–169600	88								
220R	169862–169990	43								
221R	170159–170605	149	NUDIX domain	pfam00293.8	54 (80/146)	NP_078631.1	Putative antimutator GTP pyrophosphohydrolase MutT; ORF78			
222L	171030–170830	67								
223R*	170894–171013	40								
224L	172370–171090	427	Papain family cysteine protease	pfam00112.8	66 (269/407)	NP_078647.1	Papain-like proteinase; ORF24	35 (121/338)	AF303741	CIV
225R*	171510–171647	46								
226R	172401–172751	117			46 (53/115)	NP_078646.1	ORF92			
227L	172966–172838	43								
228R	173468–173887	140								
229R	174258–174627	124								
230L	174853–174713	47								

Continued on following page

TABLE 2—*Continued*

ORF[b]	Nucleotide position	No. of amino acids	Conserved domain or signatures	CD accession no.	Homologues to LCDV-1			Homologues to other iridoviruses in *Iridoviridae*		
					% Identity of amino acids[c]	Accession no.	Predicted function and/or similarity[a]	% Identity of amino acids	Accession no.	Species
231L	176937–176209	243			58 (123/209)	NP_078737.1	ORF53			
232R*	176774–176959	62								
233L*	177093–176962	44								
234R	176981–177439	153			34 (49/141)	NP_078738.1	ORF72			
235R	177645–180923	1,093			57 (627/1086)	NP_078748.1	ORF2	26 (304/1127)	AAK37740.1	Regina ranavirus
								23 (304/1127)	NP_612298.1	ISKNV
								22 (90/409)	AF303741_	CIV
236L*	180717–80574	48								
237L	184512–181744	923	Chromosome segregation ATPases (cell division and chromosome partitioning)	COG1196.1	31 (134/432)	NP_078764.1	Putative filamentous protein; ORF10			
238R*	182066–182242	59								
239R	184871–186256	462			31 (67/212)	NP_078629.1	ORF54			
240L*	185613–185331	94								

[a] The ORF numbers of LCDV-1 are from GenBank (accession no. NC_001824).
[b] Asterisks indicate that the ORFs are not likely to represent viral genes because they overlap other large ORFs.
[c] Percentage of residues identical to those of the homologous protein or domain in the protein deduced from the ORF.

in Table 2, these ORFs encode polypeptides ranging from 40 to 1,193 amino acids. The analysis of the coding strategy of the 240 potential ORFs revealed 176 largely nonoverlapping ORFs that are likely to represent putative viral genes. As shown in Table 1, the numbers of total potential ORFs and putative genes are related to the sizes of the genomes of these characterized iridoviruses. The percent coding densities and the average lengths of ORFs for the five sequenced iridoviruses were analyzed and compared. As shown in Table 1, the percent coding density of LCDV-C is 67% and is the lowest among the iridoviruses. Moreover, the average length of each ORF in the LCDV-C genome is 702 bp, also the smallest among the iridoviruses. The unusual low coding density may be related to the presence of large noncoding regions within the gene organization and structure of LCDV-C. In the sequenced iridoviruses, the coding densities of lymphocystiviruses LCDV-1 and LCDV-C are all low and LCDV-C contains a large number of repeated sequences, which are predominantly concentrated in the gaps between two neighbor ORFs. For example, the longest gap is up to 1,895 bp and is located between ORF086L and ORF087L (Fig. 1). Thus, the LCDV-C low coding density is consistent with the high degree of large noncoding regions.

Figure 1 shows a linear map of the 176 largely nonoverlapping ORFs and their sizes, positions, and orientations in the LCDV-C genome. In the 176 putative genes, 103 genes have significant homology to the corresponding ORFs of LCDV-1, but there are still 73 potential genes that were not found in LCDV-1 and other iridoviruses (Fig. 1; Table 2). Among the 73 genes, it was found that 8 genes, ORF002L, ORF011L, ORF016L, ORF047R, ORF051L, ORF058L, ORF209R, and ORF216L, contained coding sequences for conserved domains of other cellular proteins (Table 2). For example, ORF002L contains the coding sequence for the caspase recruitment domain involved in apoptotic signaling. ORF016L contains the coding sequence for tumor necrosis factor receptor domains. ORF209R and -216L contain the coding sequences for an N-terminal domain of cell division protein 48 (CDC48) and a collagen triple-helix repeat. ORF011L, ORF047R, and ORF058L may encode thymidylate synthase, a site-specific recombinase, and a transmembrane receptor, respectively (Table 2). Interestingly, the protein product deduced from ORF051L (Table 2) is highly related to reverse transcriptase (RVT), and the C-terminal region from amino acid 191 to 446 has 26.3% identity to the consensus 200-amino-acid sequence of RVT (CD accession no. pfam00078.11, RVT) (21). Furthermore, there are 65 novel genes that do not show any significant homology with the sequences in public databases (Table 2).

Repeated sequences. Searching by the program GeneQuest of the DNASTAR software package revealed a large number of tandem and overlapping direct repeated and inverted repeated sequences in the LCDV-C genome. Although they are distributed randomly, two concentrated regions of direct repeated sequences were discovered. The first concentrated region is located from bp 1 to 530 of the genome. In the 530 bp of sequence, there are eight almost identical repeated sequences. Each repeat is composed 66 bp. As shown in Fig. 2, only six nucleotide changes occur in the first seven repeats. In the eighth repeat, the first 55-bp segment is also identical to those of the first seven repeats. And each repeat sequence

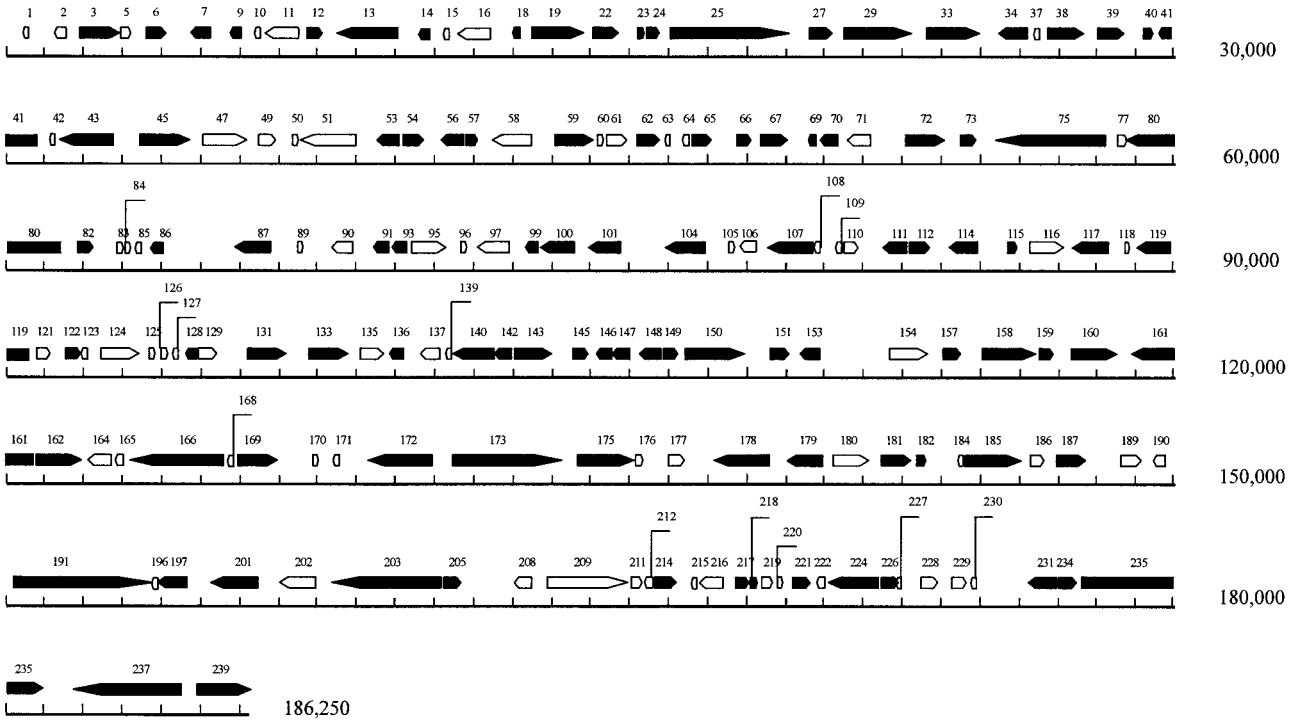

FIG. 1. Genomic organization of the LCDV-C. Arrows, locations of 176 potentially putative genes with respect of their sizes, positions, and orientations. The scale is in kilobase pairs. Black arrows, ORFs that are homologous to the potentially putative genes of LCDV-1; white arrows, potentially novel genes that were not found in LCDV-1 and other iridoviruses.

includes three short AAAGAA repeated sequences (Fig. 2). The second concentrated region, located from 124334 to 124755, includes 10 repeated sequences, and each repeat consists of 36 bp. In addition, the 52-bp repeated sequence TATATATATA TA. . .was observed at positions 25336 to 25387. Furthermore, other short repeated sequences were found dispersed all over the genome. For example, there are 73 copies of the 12-bp direct repeated sequence TTAACCCTTTAA in the genome, and 95% of them are located in the noncoding region.

In previous studies, some repeated sequences have been found in certain regions of several iridoviruses, such as FV3, LCDV-1 (27), CIV (7, 8), RSIV (35), and ISKNV (13), but the extensive and concentrated repeat sequences were observed only in the LCDV-C genome. He et al. revealed a complex cluster of multiple tandem and overlapping direct repeated sequences of 496 bp at positions 23273 to 23768 in the complete genome of ISKNV (13), but the characterization and function were unknown.

Relatedness of LCDV-C gene products to other proteins in databases. The comparison of amino acid sequences deduced from the LCDV-C ORFs with entries in protein databases led to the identification of several kinds of functionally characterized proteins in other species. These proteins included some enzymes involved in virus replication, transcription, and modification, such as DNA polymerase (ORF203L), RNA-dependent DNA polymerase (ORF051L), DNA-directed RNA polymerase (ORF115R and ORF191R), DNA methyltransferase (ORF086L), RNA polymerase (ORF025R), site-specific recombinase (ORF047R), ribonucleotide reductase (ORF041L and ORF172L), helicase (ORF75L), deoxynucleoside kinase (ORF027R), thymidylate synthase (ORF011L), protein kinase (ORF013L, ORF045R, ORF166L, ORF175R, and ORF178L), phosphatase (ORF148L), acetyl-coenzyme A hydrolase (ORF161L), and papain-like proteinase (ORF224L) (Table 2). Some of the viral proteins that might be involved in virus-host interaction were also identified from LCDV-C ORFs by significant amino acid sequence homology, such as tumor necrosis factor receptor (ORF016L), β-hydroxysteroid dehydrogenase (ORF003R), membrane-bound metallopeptidase (ORF033R), histone-like transcription factor (ORF054R), ATPase (ORF080L, ORF209R, and ORF237L), transmembrane receptor (ORF058L), and caspases (ORF002L) (Table 2). Just as for other sequenced iridoviruses (13, 14, 16, 17, 18, 33), the majority of these enzymes for LCDV-C represent homologues of cellular enzymes involved in virus replication and transcription and are shared by all iridoviruses (Table 3). Since iridoviruses form a viromatrix in cytoplasm and since their replication, transcription, and nucleotide metabolism main-

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TTGATCTAAAGAAACTTTAGAGGAAGCGTTGAAAGAAGTAGATCTATCGGCTAAGGTTAAAGAAGGATTGAT  72
      CTAAAGAAACTTTAGAGGAAGCGTTGAAAGAAGTAGATCTATCGGCTAAGGTTAAAGAAGGATTGAC 139
      CTAAAGAAACTTTAGAGGAAGCGTTGAAAGAAGTAGATCTATCGGCTAAGGTTAAAGAAGGATTGA  205
      CTAAAGAAACTTTAGAAGAAGCGTTGAAAGAAGTAGATCTATCGGCTAAGGTTAAAGAAGGATTGA  271
      CTAAAGAAACTTTAGAAGAAGCGTTGAAAGAAGTAGATCTATCGGCTAAAGTTAAAGAAGGATTGA  337
      CTAAAGAAACTTTAGAGGAAGCGTTGAAAGAAGTAGATCTATCGGCTAAAGTTAAAGAAGGATTGA  403
      CTAAAGAAACTTTAGAAGAAGCGTTGAAAGAAGTAGATCTATCGGCTAAAGTTAAAGAAGGATTGA  469
      CTAAAGAAACTTTAGAGGAAGCGTTGAAAGAAGTAGATCTATCGGCTAAGGTTAACGATACAACAG  535
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FIG. 2. Repeated sequences of bp 1 to 530 in the LCDV-C genome. There are eight direct repeated sequences with 66 bp, and each repeat sequence includes three short AAAGAA repeated sequences (boxes). The individual changed nucleotides and different nucleotides beyond the repeats are in boldface.

TABLE 3. The common genes involved in virus replication and transcription in the LCDV-C, LCDV-1, TFV, and ISKNV genomes and their ORF numbers

Function	Protein(s)	ORF for:			
		LCDV-C	LCDV-1[a]	TFV[b]	ISKNV[b]
DNA repication, modification and processing	DNA polymerase, DdDP	203L	ORF5	63R	19R
	DNA methyltransferase, DMet	86L	ORF51	89R	46L
	Helicase	75L	ORF4	9L, 56L	63L
	XPG/RAD2-type nuclease	169R	ORF34	101R	27L
Transcription of DNA	Subunit 1 of DdRP I	191R	ORF1	8L	28L
	DdRP II	25R	ORF3	65R	34R
	RNase III	187R	ORF44	85L	87R
	RBRD	41L	ORF26	71L	24R

[a] The National Center for Biotechnology Information-derived ORF numbers (GenBank accession no. NC_001824) do not correspond to the published LCDV-1 ORF numbers. They are consistent with the numbers in Table 2, column Homologues to LCDV-1.
[b] Indicates the published ORF numbers in references 13 and 14.

ly occur outside of the nucleus (42), they must establish their own replication and transcription machinery (18). Further studies on these shared genes, therefore, have significant implications for understanding the evolution and phylogeny of iridoviruses.

Comparison of LCDV-C to LCDV-1. A search of the GenBank database with the 176 individual ORFs revealed 103 homologues to those in the LCDV-1 genome (Fig. 1), accounting for 58.5% of the LCDV-C ORFs. However, comparison of the genome organizations, i.e., the putative gene orders, revealed less similarity between LCDV-C and LCDV-1. The most similar sequence between LCDV-C and LCDV-1 was located at positions 15055 to 25423 (~11 kb). It includes eight ORFs and shows 68% identity of nucleotide sequences with those of LCDV-1. Although some similarity between putative gene products of LCDV-C and the corresponding viral proteins of LCDV-1 was revealed, no whole colinearity was detected when the ORF arrangements and the coding strategies of the LCDV-C and LCDV-1 genomes were compared. The significant differences between LCDV-C and LCDV-1 genomes in gene organization and gene order are similar to those between vertebrate fish LCDV-1 and invertebrate insect CIV (18). The data suggest that there have been a large number of genetic rearrangements between LCDV-C and LCDV-1 and that the rearrangements might be of high complexity.

During the last decades, lymphocystis diseases throughout the world have been extensively described (34) and have raised serious economic problems in modern aquaculture, fish farming, and wildlife fish. In recent years, many new iridovirus-like pathogens have been isolated from over 100 different species of fish and other cold-blooded vertebrates worldwide (4, 10, 45). Indeed, LCDV and iridovirus-like pathogens vary worldwide with respect to host range and virulence, but intraspecific variation between them has been less extensively characterized. The currently studied LCDV-C was isolated in China from cultured flounder (*Paralichthys olivaceus*) with lymphocystis disease (31, 46). LCDV-C and LCDV-1 have related hosts (LCDV-1 was isolated from the flounder *Platichthys flesus*), but their geographical and temporal distributions are very different. Obviously, the significant difference in genome organization between LCDV-C and LCDV-1 suggests that such genomic differences might exist in other isolates of fish. For this reason, more work on comparative genome analysis of LCDV and other unclassified iridovirus-like isolates from distinct sources remains to be done. Recently, Goldberg et al. (11) explored intraspecific strain variation within an emerging iridovirus of North American warm-water fishes, largemouth bass virus, by amplified fragment length polymorphism analysis and revealed that the most virulent viral strain replicated to the highest level in fish. As suggested by Jakob and Darai (18), a substantial revision of the taxonomy of LCDV isolates and other iridoviruses based on molecular anatomy and phylogeny is required.

Relationship of LCDV-C to other iridoviruses and its taxonomic position. The highest homologies were detected between putative gene products of LCDV-C and the corresponding viral proteins of LCDV-1, but some important genes involved in virus replication, transcription, and modification in the LCDV-C genome have been identified previously in three other vertebrate iridovirus genomes that were completely sequenced, including the LCDV-1 (32), TFV (14), and ISKNV (13) genomes. As shown in Table 3, these genes included those encoding DNA polymerase, DNA methyltransferase, helicase, XPG/RAD2-type nuclease, subunit 1 of DNA-dependent RNA polymerase (DdRP), DdRP II, RNase III, and ribonucleotide reductase (RBRD).

The LCDV-C MCP is encoded by ORF043L and is composed of 459 amino acids (Table 2). It presents the highest identity to those of LCDV-1 and other iridoviruses among the putative gene products of LCDV-C. Homology analysis showed that the MCPs of LCDV-1 (33), CIV (16), TFV (14), FV3 (20), EHNV (22), BIV (3), RSIV (23), and ISKNV (13) had 87.6, 53.0, 51.1, 50.9, 50.7, 50.7, 49.0, and 49.2% identity to that of LCDV-C, respectively. Based on the multiple alignments of amino acid sequences of nine complete MCPs, a phylogenetic tree was constructed. As shown in Fig. 3, the nine iridoviruses are divided into four groups, the lymphocystiviruses LCDV-C and LCDV-1; the insect iridoviruses, including CIV; the ranaviruses, including FV3, BIV, TFV, and EHNV; and the unassigned viruses ISKNV and RSIV. Interestingly, LCDV-C and LCDV-1 are clustered together, but their amino acid identity is much less than that in the other three clusters. Recently, Jakob and Darai (18) drew the conclusion that a cricket iridovirus (CrIV) isolate and CIV are not different species because of the high identity (97.9%) of their MCP amino acid sequences and

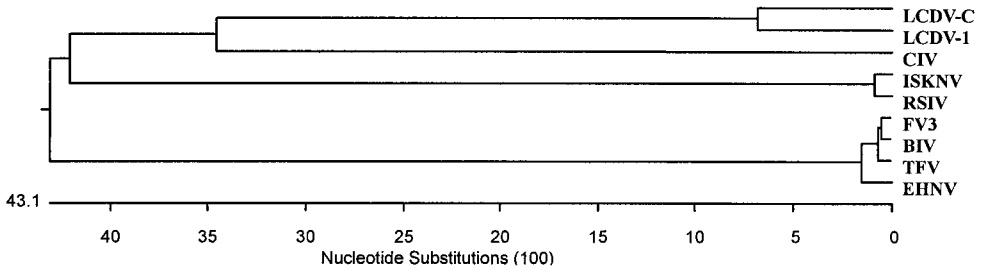

FIG. 3. Phylogenetic tree based on the multiple alignments of the amino aced sequences of the MCPs of iridoviruses. The GenBank accession numbers for the virus nucleotide sequences are as follows: LCDV-1, AAC24486; CIV, AAK82135; ISKNV, AAL98730; RSIV, BAC66968; FV3, Q67473; TFV, AF389451; EHNV, AA032315; BIV, AY187046.

considered CrIV a variant or a strain of CIV. The MCPs of FV3, TFV, ENHV, and BIV have over 96.8% identity (Fig. 3), suggesting that these viruses might also be different variants of the same species. And the identities of MCPs of ISKNV and RSIV were also found to be up to 98.2%. However, LCDV-C was identified to be the Chinese LCDV variant on the basis of the infection symptoms (31, 40) and viral morphology (46), but the MCPs of LCDV-C and LCDV-1 have only 87.6% identity, and there are significant differences between their genome sizes (Table 2) and gene organizations (Fig. 1). The unexpected levels of divergence between their genomes in size, gene organization, and gene product identity suggest that LCDV-C and LCDV-1 shouldn't belong to a same species and that LCDV-C should be considered a separate species, different from LCDV-1.

LCDV-C is the second LCDV isolate whose complete genomic sequence has been determined since the first complete genome of LCDV was sequenced from the LCDV-1 isolate in 1997 (33). Up to now, more than 100 new iridovirus-like isolates have been reported from over 100 different species of fish in seawater and freshwater worldwide (34). Of the numerous virus isolates, only two isolates have been completely sequenced, and a great number of divergences between them have been revealed. Obviously, a handicap for further analysis is the lack of genome sequence information for other iridovirus-like isolates (18). Therefore, the significant divergences between LCDV-C and LCDV-1 draw our attention to the different iridovirus-like isolates. The detailed molecular anatomy and functional analyses of these different iridovirus-like isolates will provide more novel and distinct knowledge about their relationship and taxonomic position among the iridoviruses.

ACKNOWLEDGMENTS

We thank Sun Xiu-qin for her assistance on sampling the diseased fish, and we thank members of BGI Life Tech, Beijing, for their assistance on sequencing technologies.

This study was supported by National 863 Project of China (2001AA626030 and 2002AA626010), the National Natural Science Foundation of China (30170726), the Project of Chinese Academy of Sciences (STZ-00-13, KSCX2-SW-302), and the Frontier Science Project Program of the Institute of Hydrobiology, Chinese Academy of Sciences (220301).

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